Karyotypes of Dremomys pernyi and D. pyrrhomerus (Rodentia: Sciuridae) from China

AUTHOR(S):

Motokawa, Masaharu; Cong, Haiyan; Kong, Lingming; Harada, Masashi; Wu, Yi; Li, Yuchun

CITATION:

Motokawa, Masaharu ...[et al]. Karyotypes of Dremomys pernyi and D. pyrrhomerus (Rodentia: Sciuridae) from China. Mammal Study 2017, 42(1): 45-49

ISSUE DATE:

2017-03-01

URL:

http://hdl.handle.net/2433/250821

Karyotypes of Dremomys pernyi and D. pyrrhomerus (Rodentia: Sciuridae) from China

Authors: Motokawa, Masaharu, Cong, Haiyan, Kong, Lingming, Harada, Masashi, Wu, Yi, et al.

Source: Mammal Study, 42(1) : 45-49

Published By: Mammal Society of Japan

URL: https://doi.org/10.3106/041.042.0105

Mammal Study 42: 45–49 (2017)

Short communication

Karyotypes of *Dremomys pernyi* and *D. pyrrhomerus* (Rodentia: Sciuridae) from China

Masaharu Motokawa[1], Haiyan Cong[2], Lingming Kong[2], Masashi Harada[3], Yi Wu[4] and Yuchun Li[2,*]

[1] *The Kyoto University Museum, Kyoto University, Kyoto 606-8501, Japan*
[2] *Marine College, Shandong University, Weihai 264209, China*
[3] *Laboratory Animal Center, Graduate School of Medicine, Osaka City University, Osaka 545-8585, Japan*
[4] *College of Life Sciences, Guangzhou University, Guangzhou 510006, China*

Abstract. Karyotypes of *Dremomys pernyi* from Kanding, Sichuan Province, China and *D. pyrrhomerus* from Nanling, Guangdong Province, China were examined. The karyotype of *D. pernyi* was of 2n = 40 and FNa = 72, while that of *D. pyrrhomerus* was of 2n = 38 and FNa = 70. Karyotype of *D. pernyi* from Kanding was different in 2n and FNa from reported karyotype of conspecific population from Taiwan, and it is suggested that the Taiwan population represents a distinct species *D. owstoni* from *D. pernyi*. The difference between karyotypes of *D. pernyi* and *D. pyrrhomerus* may involve at least a Robertsonian rearrangement and a heterochromatin addition.

Key words: *Dremomys*, karyotype, Sciuridae, Taiwan, taxonomy.

The genus *Dremomys* is plain long-nosed squirrels of Asia comprising six species (Thorington and Hoffmann 2005; Thorington et al. 2012). Five species, including *D. pyrrhomerus* (Thomas, 1895), *D. rufigenis* (Blanford, 1878), *D. pernyi* (Milne-Edwards, 1867), *D. gularis* Osgood, 1932, and *D. lokriah* (Hodgson, 1836), are distributed in China and Indochina region, while another species *D. everetti* is known from Borneo (Hoffmann and Smith 2008; Thorington et al. 2012). Species taxonomy and boundary is still unclear, especially among *D. pyrrhomerus*, *D. rufigenis*, and *D. pernyi*. Oshida et al. (2003) reported a karyotype of *D. pernyi* from Taiwan and discussed the karyotype differences between Taiwan and Yunnan populations reported by Wang et al. (1980), and noted that the Taiwan *D. pernyi* population is more similar to *D. rufigenis* from Vietnam with a diploid chromosome number (2n) of 38 and a fundamental number of autosomal arms (FNa) of 68, reported by Nadler and Hoffmann (1970). Although 2n is similar between *D. pernyi* from Taiwan and *D. rufigenis* from Vietnam, the karyotypes were found to be different in the published figures; there were seven subtelocentric pairs in Vietnam *D. rufigenis* population, and nine pairs in Taiwan *D. pernyi* population. Among *Dremomys* species, the taxonomic status of *D. pyrrhomerus* has also been confused; it had been considered conspecific with *D. rufigenis* (e.g., Corbet and Hill 1992). While, these two species are currently considered different species due to their sympatric distribution (Thorington and Hoffmann 2005; Hoffmann and Smith 2008), but their distribution boundary between species is still unclear. Karyological study of *Dremomys* species is expected for the revision of species taxonomy of the genus. In the present study, we report karyotypes of two *Dremomys* species from China and discuss the taxonomic implications of *D. pernyi*, *D. pyrrhomerus*, and *D. rufigenis*.

Materials and methods

One male specimen of *D. pernyi* (G10138) was collected in August 2010, from Kangding, Ganzi Tibetan Autonomous Prefecture, Sichuan Province, China; and one male specimen of *D. pyrrhomerus* (G12185) was collected in August 2012, from Nanling, Guangdong Province, China (Fig. 1). The voucher specimens were deposited in the Key Laboratory of Conservation and

*To whom correspondence should be addressed. E-mail: li_yuchun@sdu.edu.cn

Fig. 1. Map of China showing the sampling localities of *Dremomys pernyi* (A) and *D. pyrrhomerus* (B).

Application in Biodiversity of South China, Guangzhou University (G10138) and Marine College, Shandong University, Weihai (G12185).

Cytological preparations were made from tail and/or lung tissue culture cells using the standard air-drying method as described by Harada and Yosida (1978). G-band and C-band stainings were accomplished with the methods of Seabright (1971) and Sumner (1972), respectively. Terminology for chromosomes follows Levan et al. (1964): metacentric, submetacentric, subtelocentric, and acrocentric. The 2n and FNa values for each species were calculated.

Results

The karyotype of *D. pernyi* (Fig. 2) was of 2n = 40 (FNa = 72) chromosomes consisting of eight large- to small-sized meta- or submetacentric pairs (nos. 1–8), nine large- to small-sized subtelocentric pairs (nos. 9–17), and two medium- to small-sized acrocentric pairs (nos. 18–19) in autosomes and a medium-sized submetacentric X chromosome and a small-sized subtelocentric Y chromosome. In an acrocentric pair (no. 19), there was secondary constriction at the proximal region of long arms as shown with an arrow (Fig. 2A). Short arm of a subtelocentric pair (either of nos. 10–13) was stained with C-band and considered heterochromatic (Fig. 2C, shown with an arrow). In addition, the autosome pairs of nos. 1–3 and no. 9 had small centromeric C-bands; the short arms of pairs 15–17 were entirely heterochromatic; telomeric C-bands were detected either on the short arm or the long arm, or on both arms of pairs nos. 1–4, 9, 18 and 19; the Y chromosome was entirely heterochromatic (Fig. 2C).

The karyotype of *D. pyrrhomerus* (Fig. 3) was of 2n = 38 (FNa = 70) chromosomes consisting of 12 large- to small-sized meta- or submetacentric pairs (nos. 1–12), five large- to small-sized subtelocentric pairs (13–17), and one small-sized acrocentric pair (no. 18) in autosomes, and a medium-sized submetacentric X chromosome and a small-sized subtelocentric Y chromosome. In a medium-sized metacentric pair (no. 8), there was secondary constriction at the proximal region of short arms as shown with an arrow (Fig. 3A). The autosome pairs of nos. 4 and 8 had small centromeric C-bands; the short arms of pairs nos. 3, 8, 10, 11, 15, and 17 were entirely heterochromatic; telomeric C-bands were detected either on the short arm or the long arm, or on both arms of pairs nos. 2–4, 8, 13–16, and X chromosome; the long arms of Y chromosome was heterochromatic (Fig. 3C)

G-band comparison detected chromosome pair matching between *D. pernyi* and *D. pyrrhomerus* (Fig. 4). We found that *D. pernyi* no. 18 long arm and no. 13 long arm

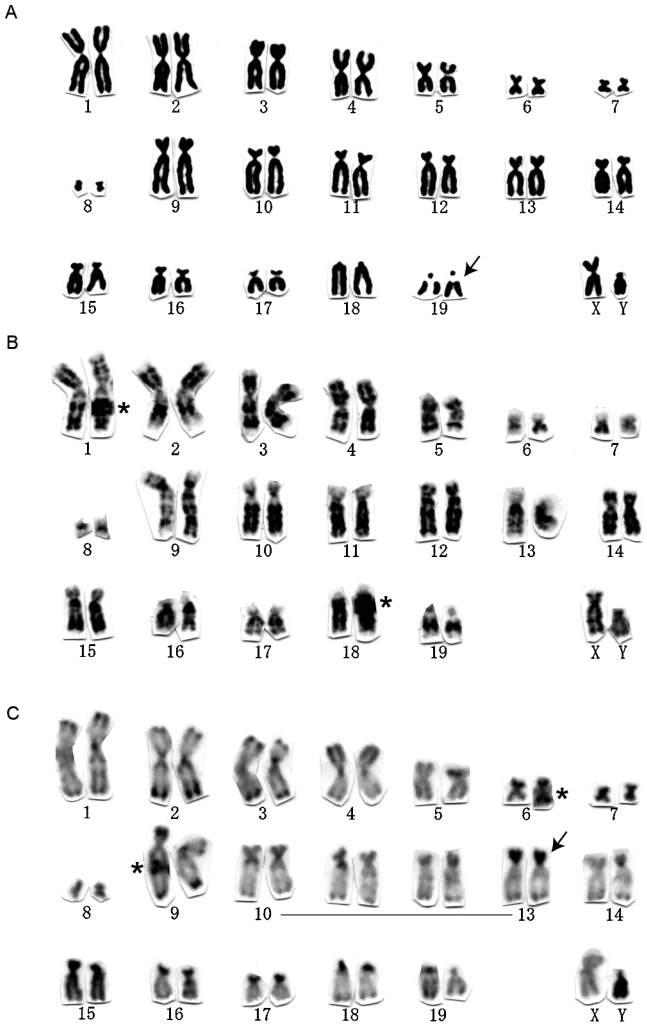

Fig. 2. Conventionally stained (A), G-banded (B), and C-banded (C) karyotypes of *Dremomys pernyi* from China. Arrows indicate the secondary constriction (B) and heterochromatic arm (C). Asterisks show the overlapping of chromosome arms.

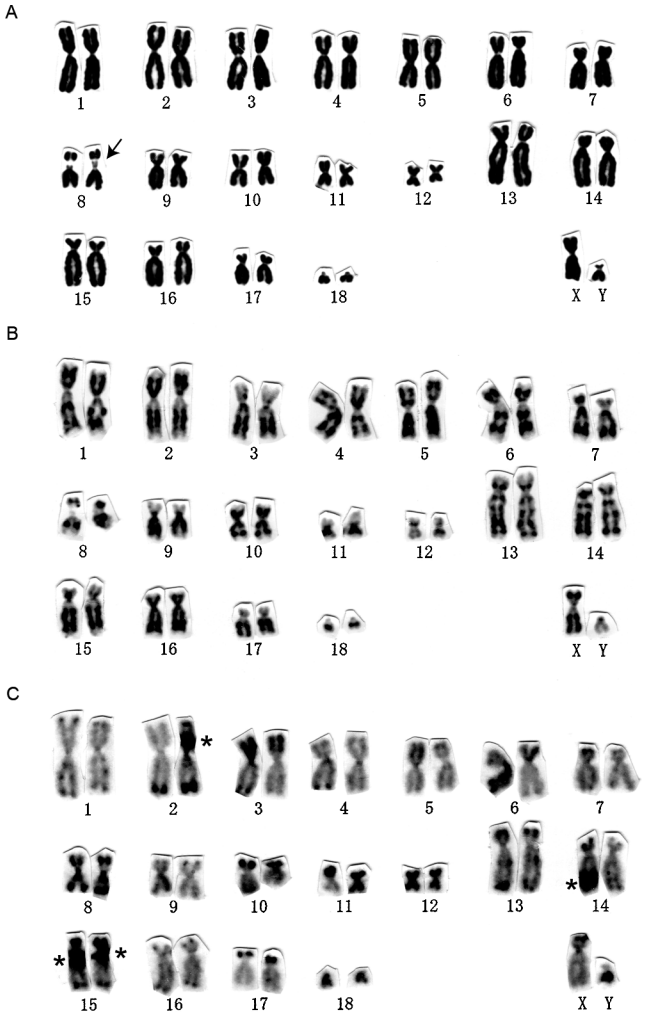

Fig. 3. Conventionally stained (A), G-banded (B), and C-banded (C) karyotypes of *D. pyrrhomerus* (B) from China. Arrow indicates the secondary constriction. Asterisks show the overlapping of chromosome arms.

correspond to *D. pyrrhomerus* no. 5 long arm and short arm, respectively (Fig. 4, indicated in box). *Dremomys pernyi* no. 19 long arm corresponded with *D. pyrrhomerus* no. 8 long arm, while we could not detect the homology of *D. pyrrhomerus* no. 8 short arm. Meta- or submetacentric *D. pernyi* no. 8 pair corresponded with acrocentric *D. pyrrhomerus* no. 18 pair; and subtelocentric *D. pernyi* no. 11 pair corresponded with metacentric *D. pyrrhomerus* no. 3 pair; while detailed chromosome rearrangements could not be detected in these pairs.

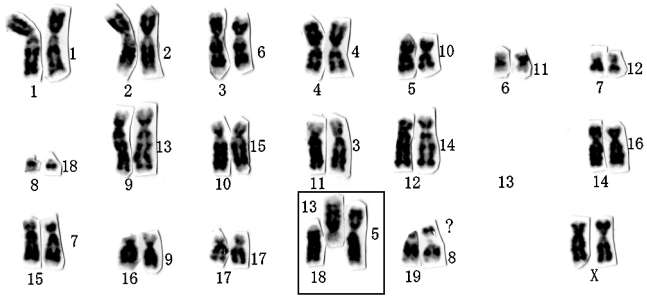

Fig. 4. G-band matching between *D. pernyi* (left) and *D. pyrrhomerus* (right).

Discussion

Dremomys pernyi was originally described from Mouping (currently Baoxing) in Sichuan Province, China, and it is distributed in northeast India, north Burma, north Vietnam, and south China including Taiwan (Thorington and Hoffmann 2005). The collection site of the present

study, Kangding, is less than 100 km from the type locality (Fig. 1). The karyotypes of *D. pernyi* from Kangding was 2n = 40 and FNa = 72, and from Yunnan (as subspecies *D. pernyi flavior*; 2n = 40 and FNa = 70) reported by Wang et al. (1980) have same 2n, but different FNa. Such chromosome difference was due to the number of medium-sized acrocentric pairs: two pairs in Kanding and three pairs in Yunnan. Further study is necessary for precise understanding of this geographic chromosomal variation.

The present karyotype is, however, different from the karyotype of the conspecific Taiwan population carrying 2n = 38 and FNa = 68 (Oshida et al. 2003). In addition to the differences in 2n and FNa, an acrocentric pair from Kanding had secondary constriction on proximal part of long arm (Fig. 2A, no. 19), while the similar sized acrocentric pair from Taiwan had secondary constriction on the proximal part of short arms with developing satellite on the terminal region of short arms (Oshida et al. 2003, no. 17). The heterochromatic short arms of a subtelocentric pair exist in the former (Fig. 2C, no. 13), while they are absent in the latter (Oshida et al. 2003). Although we could not directly compare G-band patterns between karyotypes from Kangding and Taiwan, karyological differences with different FNa values are not responsible for Robertsonian rearrangements, and more complicated chromosome rearrangements of above-mentioned and of possible additional tandem fusions/fissions might have established postmating cytological reproductive isolation (King 1993) between the two populations. The karyotype of *D. pernyi* in this study is from very close to the type locality and could be considered representative of the species. The Taiwan population has sometimes been considered a distinct subspecies, *D. p. owstoni*, which was originally described from Mt. Alishan in Taiwan (Thomas 1908). In this paper, we suggest the Taiwan population to be recognized a distinct species *D. owstoni* from *D. pernyi*, and further morphological analysis to verify the distinctness between *D. pernyi* and *D. owstoni* will be conducted.

To our knowledge, this is the first report of the karyotype of *D. pyrrhomerus*. It resembles the karyotype of *D. rufigenis* from Vietnam with 2n = 38 (Nadler and Hoffmann 1970), but there are some differences in conventionally stained karyotypes between two species in this study in addition to different interpretation for the numbers of M/SM, ST, and A (Table 1). Difference in FNa (70 in *D. pyrrhomerus* and 68 in *D. rufigenis*) is likely to be related to the possible chromosomal rearrangement between medium-sized metacentric pair with a secondary constriction (no. 8) in *D. pyrrhomerus* and the acrocentric pair developing secondary constriction on the proximal part of short arms and prominent satellite on terminal region of short arms in *D. rufigenis* reported by Nadler and Hoffmann (1970). *Dremomys rufigenis* has been considered a sister species of *D. pyrrhomerus* based on the mitochondrial cytochrome *b* gene phylogeny (Li et al. 2008) and similarity in skull morphometrics (Li 2010). In the past, *D. pyrrhomerus* was sometimes considered conspecific with *D. rufigenis* (e.g., Corbet and Hill 1992); but the two species showed mostly separate but close distributions involving sympatric localities (Zhang et al. 1997; Thorington and Hoffmann 2005). This study may suggest the occurrence of reproductive isolation between the two species due to chromosomal rearrangement; and future study for G-band and C-band karyotypes for *D. rufigenis* is expected for detailed comparison between these two species.

G-band and C-band comparisons between *D. pernyi* and *D. pyrrhomerus* showed well matching of homologous arms. Notable chromosome rearrangements were found between *D. pernyi* nos. 13/18 pairs and *D. pyrrhomerus* no. 5 pair. We suggest that *D. pernyi* karyotype might have been derived from the karyotype similar to *D. pyrrhomerus*, with the Robertsonian fission of *D. pyrrhomerus* no. 5 pair, producing *D. pernyi* nos. 13 and 18 pairs; and subsequent heterochromatin addition in *D. pernyi* no. 13 pair to form short arms. Future studies

Table 1. Karyotype composition of *Dremomys* species

Species	2n	FNa	Autosomal pair			X	Y	Reference
			M/SM	ST	A			
D. pernyi (Sichuan)	40	72	8	9	2	SM	ST	This study
D. pernyi (Yunnan)	40	70	8	8	3	SM	SM	Wang et al. (1980)
D. pernyi (Taiwan)	38	68	7	9	2	SM	A	Oshida et al. (2003)
D. pyrrhomerus (Guangdong)	38	70	12	5	1	SM	ST	This study
D. rufigenis (Vietnam)	38	68	9	7	2	SM	SM	Nadler and Hoffmann (1970)

for G-band and C-band karyotypes for other *Dremomys* species including *D. owstoni* in Taiwan are expected to explore detailed chromosome evolution in the genus *Dremomys*.

Acknowledgments: This study was supported by grants from the NSFC Major International (Regional) Joint Research Project Grant (No. 31110103910), JSPS Core-to-Core Program B. Asia-Africa Science Platforms, and JSPS Grant-in-Aid for Scientific Research (No. 26304009). We thank two anonymous reviewers for useful comments to improve the manuscript.

References

Corbet, G. B. and Hill, J. E. 1992. The Mammals of the Indomalayan Region: A Systematic Review. Oxford University Press, Oxford, 488 pp.

Harada, M. and Yosida, T. H. 1978. Karyological study of four Japanese *Myotis* bats (Chiroptera, Mammalia). Chromosoma 5: 283–291.

Hoffmann, R. S. and Smith, A. T. 2008. Family Sciuridae. In (Smith, A. T. and Xie, Y., eds.) A Guide to the Mammals of China, pp. 172–196. Princeton University Press, Princeton.

King, M. 1993. Species Evolution; The Role of Chromosome Change. Cambridge University Press, Cambridge, 336 pp.

Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52: 201–220.

Li, S. 2010. A cladistic phylogeny of the plain long-nosed squirrels (Sciuridae, *Dremomys*) from the mainland of southeastern Asia based on morphological data. Acta Theriologica Sinica 30: 119–126.

Li, S., Yu, F., Yang, S., Wang, Y., Jiang, X., McGuire, P. M., Feng, Q. and Yang, J. 2008. Molecular phylogeny of five species of *Dremomys* (Rodentia: Sciuridae), inferred from cytochrome *b* gene sequences. Zoologica Scripta 37: 349–354.

Nadler, C. F. and Hoffmann, R. S. 1970. Chromosomes of some Asian and South American squirrels (Rodentia: Sciuridae). Experientia 26: 1383–1386.

Oshida, T., Lee, J. K., Yuan, S. L. and Lin, L.-K. 2003. A preliminary note on banded karyotypes of the Perny's long-nosed squirrel *Dremomys pernyi* (Mammalia, Rodentia) from Taiwan. Caryologia 56: 171–174.

Seabright, M. 1971. A rapid banding technique for human chromosomes. Lancet 2: 971–972.

Sumner, A. T. 1972. A simple technique for demonstrating centromeric heterochromatin. Experimental Cell Research 75: 304–306.

Thomas, O. 1908. On the generic position of the groups of squirrels typified by "*Sciurus*" *berdmorei* and *pernyi*. Journal of Bombay Natural History Society 18: 244–249.

Thorington, R. W., Jr. and Hoffmann, R. S. 2005. Family Sciuridae. In (Wilson, D. E. and Reeder, D. M., eds.) Mammal Species of the World: A Taxonomic and Geographic Reference Third edition, pp. 754–818. The Johns Hopkins University Press, Baltimore.

Thorington, R. W., Jr., Koprowski, J. L., Steele, M. A. and Whatton, J. F. 2012. Squirrels of the World. The Johns Hopkins University Press, Baltimore, 459 pp.

Wang, Y., Li, S., Li, C., Wang, R. and Liu, G. 1980. Karyotypes and evolution of three species of Chinese squirrels (Sciuridae Mammalia). Zoological Research 1: 501–521 (in Chinese with English abstract).

Zhang, R., Jin, S., Quan, Q., Li, S., Ye, Z., Wang, F. and Zhang, M. 1997. Distribution of Mammalian Species in China. China Forestry Publishing House, Beijing, 280 pp.

Received 26 July 2015. Accepted 5 October 2016.
Editor was Tatsuo Oshida.